EVALUATION OF LEPTIN LEVELS IN GINGIVAL CREVICULAR FLUID DURING ORTHODONTIC TOOTH MOVEMENT

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CERTIFICATE

This is to certify that the dissertation entitled "**Evaluation of Leptin Levels In Gingival Crevicular Fluid During Orthodontic Tooth Movement"** done by **Dr. Joseph. A,** post graduate student (M.D.S), Orthodontics (Branch V), Tamil Nadu Govt. Dental College and Hospital, Chennai, submitted to the Tamil Nadu Dr.M.G.R.Medical University in partial fulfilment for the M.D.S. degree examination **(April 2011)** is a bonafide research work carried out by him under my supervision and guidance.

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DECLARATION

I, **Dr. Joseph.A**, do hereby declare that the dissertation titled **"Evaluation of Leptin Levels In Gingival Crevicular Fluid During Orthodontic Tooth Movement"** was done in the Department of Orthodontics, Tamil Nadu Government Dental College & Hospital, Chennai 600 003. I have utilized the facilities provided in the Government Dental College for the study in partial fulfilment of the requirements for the degree of Master of Dental Surgery in the specialty of Orthodontics and Dentofacial Orthopaedics (Branch V) during the course period 2008-2011 under the conceptualization and guidance of my dissertation guide, Professor **Dr.M.C. Sainath, MDS.**

I declare that no part of the dissertation will be utilized for gaining financial assistance for research or other promotions without obtaining prior permission from the Tamil Nadu Government Dental College & Hospital.

I also declare that no part of this work will be published either in the print or electronic media except with those who have been actively involved in this dissertation work and I firmly affirm that the right to preserve or publish this work rests solely with the prior permission of the Principal, Tamil Nadu Government Dental College & Hospital, Chennai 600 003, but with the vested right that I shall be cited as the author(s).

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INTRODUCTION

Orthodontic tooth movement is based on force induced periodontal ligament and alveolar bone remodeling. Mechanical stimuli exerted on a tooth causes inflammatory response in periodontium. Inflammatory mediators are released that trigger the biological processes associated with alveolar bone resorption and deposition. The knowledge of two possible control elements namely bioelectric signal and chemical signal are mandatory for better understanding of the physiologic response of the teeth against sustained pressure. Hence it is necessary to consider the biologic control mechanism that leads from mechanical stimulus of sustained force application to the response of orthodontic tooth movement.

Cytokines are one among the local biochemical mediators of tooth movement and are secreted mainly by adipocytes[2] and also by mononuclear cells and leukocytes.[17] Cytokines can provoke the synthesis and secretion of numerous substances that form the molecular basis for cell-to-cell communication, including prostaglandins (PGs) and

growth factors, thus interacting directly or indirectly with bone cells [17] Cytokines are extracellular signaling proteins that act on nearby target cells in low concentration. Cytokines are involved in initiating, amplifying, perpetuating and resolving inflammatory responses. Cytokines have multiple biologic activities and they are also involved in bone remodeling, resorption and or new bone deposition and thus they play an important role in tooth movement [2]. Leptin is a 16 KDa non –glycosylated polypeptide hormone and has been classified as pro inflammatory cytokine. It could be easily defined as cytokine like hormone with pleiotropic actions [65]. It was named leptin after the Greek God Leptos which means "THIN"[69]. Leptin is chiefly synthesized and secreted by adipocytes. Leptin has been reported to influence various biological mechanisms including the immune and inflammatory response, hematopoeisis, angiogenesis, bone formation and wound healing.[86]Acute infection, sepsis and wide range of inflammatory mediators increase leptin synthesis. However chronic stimulation induces a suppression of leptin synthesis.[65] Leptin orchestrates the host response to inflammatory and infectious stimuli as it

stimulates the immune response by enhancing cytokine production and phagocytosis of macrophages[22] Thus overall increase in leptin during inflammation and infection indicates leptin is a part of immune response and defense mechanism[4]. Recently it has been suggested that leptin plays a significant role in bone formation by virtue of its direct effect on osteoblast proliferation, differentiation and in prolonging the life span of human primary osteoblasts by inhibiting apoptosis.[90] Thus leptin at high concentration protects the host from inflammation and infections and maintains the bone level that is very crucial for orthodontic tooth movement.[34]

Gingival crevice fluid (GCF) is an inflammatory exudate that seeps into gingival crevices or periodontal pockets around teeth with inflamed gingiva [15] Since 1960, when it was first suggested that analysis of GCF might be a way to quantitatively evaluate the inflammatory status of gingival and periodontal tissues[11], there has been intense interest in the diagnostic potential of GCF. Recently, a number of GCF constituents have been shown to be diagnostic markers of active tissue destruction in

periodontal diseases [38] Therefore biochemical analysis of GCF provides a non- invasive model for investigating the cellular response of underlying PDL during orthodontic tooth movement.[83]

The purpose of this study was to test the levels of leptin in GCF around a moving tooth and to find if any changes in leptin level occur during Orthodontic tooth movement after applying constant continuous force.

AIM & OBJECTIVES

AIM

To evaluate the levels of leptin in gingival crevicular fluid during orthodontic tooth movement

OBJECTIVES

- To find the leptin levels in gingival crevicular fluid in normal healthy patients
- To find the leptin levels during orthodontic tooth movement in the same sample without applying retractive force
- To find the leptin levels in orthodontic during orthodontic tooth movement after applying retractive force
- To compare the above two values and to determine whether any changes in leptin level occur during orthodontic tooth movement
- To find the role of leptin as a mediator for tooth movement

REVIEW OF LITERATURE

Orthodontic tooth movement is the result of alveolar bone remodeling due to mechanical stimulus at the interface with periodontal ligament. But the important factors for effective tooth movement include patients' cell biology at bio molecular level. A perusal of literature pertaining to tooth movement indicate that researchers have investigated several factors involved in bone remodeling and the role of cytokines in gingival crevicular fluid that are said to be one of the bio-mediators of tooth movement. Very few studies have been undertaken to implicate the pivotal role of leptin as a biomarker in gingival crevicular fluid during orthodontic tooth movement.

STUDIES RELATED TO ORTHODONTIC TOOTH MOVEMENT AND BIOMARKERS IN GCF

Sandstedt (1904)[77] showed histologicaly that bone gets resorbed in area of pressure and gets deposited in areas of tension

Oppenheim (1911)[55] reported necrotic areas produced in PDL on pressure side when heavy forces were used.

Luz. C. Macapanpan, Joseph (1954)[44] studied rat dentition to explore tissue changes following tooth movement. They concentrated on early changes from (1-72 hrs) following tooth movement. They stated that not only osteoblasts and osteoclasts but also fibroblasts play an important role in repair following tooth movement.

Kaare Reitan (1964)[33] stated that light interrupted force results in direct resorption on pressure side. On the other hand when a strong continuous force is exerted root resorption occurs.

Theodre M. Trick (1967)[79] did a study to evaluate the PDL tissue response to orthodontic tooth movement by panoromix. Eventhough some Periodontists have indicated that one of the etiological factors in developing PDL disease is orthodontic treatment and orthodontic treatment is destructive to investing tissues of teeth orthodontists

have held that physiologic tooth movements with orthodontic appliance is possible.

Erickson et al (1978)[21] demonstrated that in absence of plaque orthodontic forces moving individual teeth bodily in dog not induce gingivitis. In presence of plaque similar forces are not capable of converting gingival inflammation into a destructive and progressive periodontal disease

Shanfeld, JoLynda Davidovitch (1986)[74] Conducted a study in which the objective was to extract and assay cyclic nucleotides and prostaglandins from tissues surrounding orthodontically treated canines in cats. The results demonstrated that alterations in the levels of each of these substances in tissues surrounding teeth may be brought about by long-term applications of orthodontic force in vivo.

Samuel J. Burrow, Patrick J. et al (1986)[67] did a study on the Effects of diazepam on orthodontic tooth movement and alveolar bone cAMP levels in cats. Cyclic AMP has been suggested as a possible intracellular

mediator in bone remodeling during tooth movement interestingly, although diazepam had no effect on undisturbed tissues, it lowered the cAMP levels in the periodontal tissues of orthodontically moved teeth. . On the basis of these results, it was concluded that the concentration of cAMP did not correlate with bone remodeling in this model and perhaps should not be used as an index of periodontal-tissue response during orthodontic tooth movement.

Christer Engström, Göste Granström, Birgit Thilander (1988)[13] conducted a study in which the aim was to, by histologic and new biochemical methods, to investigate the effect of orthodontic forces on the periodontal tissues in the normal and the hypocalcemic situation with secondary hyperparathyroidism. This study has shown that root resorptions were clearly related to the degradation process occurring in the vicinity of the hyaline zone and that in the hypocalcemic situation, the increase in root resorptions was related to an enhanced alveolar bone resorption.

Abbas H. Mohammed, Dimitris N. Tatakis, Rosemary Dziak (1989)[1] did a study on the role of Luekotrienes in modulating or mediating orthodontic tooth movement. They concluded that significant inhibition of tooth movement occurred beginning on day 7 in the indomethacin.

R. L. Boyd, P. J. Leggott, R. S. Quinn, W. S. Eakle, D.Chambers (1989)[60] conducted a longitudinal study and monitored periodontal status in 20 adults and 20 adolescents undergoing fixed orthodontic treatment. They also received periodontal maintenance at 3-month intervals during orthodontic treatment. Periodontal status was determined (1) at six standard sites before fixed appliances were placed (baseline), (2) at 1, 3, 6, 9, 12, and 18 months after appliances had been place, and (3) 1, 3, 6, and 12 months after appliances had been removed. During orthodontic treatment the adolescent group showed significantly more periodontal inflammation and supragingival plaque than the adults; after appliances were removed, this pattern was no longer statistically significant. For loss of attachment, there were no significant differences

among adolescents, adults with normal periodontal tissues, or adults with reduced but healthy periodontal tissues who had undergone treatment for periodontal disease.

Jan L. Wennström et al (1993)[30] did a study to evaluate the Periodontal tissue response to orthodontic movement of teeth with infrabony pockets . It was concluded that orthodontic therapy involving bodily tooth movement may enhance the rate of destruction of the connective tissue attachment at teeth with inflamed, infrabony pockets and that the risk for additional attachment loss is particularly evident when the tooth is moved into the infrabony pocket.

Lu et al (1993)[37] stated that elastomeric chains from different manufacturers vary in their properties. Longer the stretch of the elastomeric chains more the rate of decay of its force. None of the chains produce more than 180 gm of forces for more than 3 weeks. and several of the chains for less than 1 week. Starting force of these elastomeric chains were approximately around 400gm. Moreover 50% -70% of their force had decayed by 21 days.

Baty et al (1994)[9] in a comprehensive review of elastomeric chains concluded that most studies indicate a loss of 50% - 70% of force in the 1st day with only 30%-40% remaining at 3 weeks. He also reported that pre stretching of elastomeric chains in order to reduce rapid decay of force only increased the residual force at 3 weeks by 5% which in clinically insignificant.

J. Okasaki et al (1995)[29] found that chondroitin sulphate is found in all GCF samples with greater amount in periodontal disease that at control sites with a relatively healthy periodontium.

Setsuko Uematsu Et Al (1996)[73] did a study to identify and quantify Transforming Growth Factor- B1 (TGF-B1) in human gingival crevicular fluid and to investigate changes occurring during orthodontic tooth movement. The concentration of TGF_B1 was significantly higher in experimental group than the control. Results suggested that TGF_B1 is associated with bone remodeling that occurs during orthodontic tooth movement

Michael, Gregory J. King, (1996)[49] examined acid and alkaline phosphatase activities in gingival crevicular fluid (GCF) to learn whether bone turnover dynamics can be monitored in human subjects during orthodontic tooth movement. Alkaline phosphatase peaked between the first and third weeks, followed by an increase in acid phosphatase between the third and sixth weeks. The GCF phosphatase activities were assessed as functions of location on the tooth, treatment modality, duration of treatment, gingival inflammation, and plaque accumulation. The plaque index did not show a relationship to either acid or alkaline phosphatase activity on the mesial or distal in the treated groups. However, alkaline phosphatase increased with inflammation on the distal in treated groups and acid phosphatase was consistently higher on the mesial than on the distal in the treatment groups. Alternating peaks of acid and alkaline phosphatase were found in GCF of treated teeth as functions of treatment duration. It was concluded that phosphatase activities in GCF may be a useful means for monitoring tissue responses to orthodontic treatment.

Samuel et al (1998)[66] stated that existing evidence support that a nominal of 150 gm NiTi coil springs usually works well clinically.

C.-C. Tsai, Y. C. Hong, C. C. Chen (1998)[12] stated that the arachidonic acid metabolites prostaglandin E, (PGE,) and leukotriene B, (LTB,) are inflammatory mediators which are likely to be involved in the pathogenesis of periodontal disease. The objectives of this study were to measure gingival crevicular fluid (GCF) levels of PGE, LTB, and periodontal health. Results showed significant differences in the levels of PGE, and LTB, were found between patients with periodontitis, and non-periodontitis individuals. The PGE, LTB, levels were positively correlated with the clinical parameters and reduced markedly after phase1 of the periodontal treatment. The total amount and concentration of LTB, was positively correlated with the gingival index. These results indicate that the levels of PGE, correlated with the severity of the periodontal status, and the levels of LTB, correlated with gingival inflammation . Thus the data suggest that the total

amounts of PGE and LTB, may be good indicators for periodontal inflammation,

G. J. King, L. Archer, (1998)[23] stated that **d**elays in the appearance of osteoclasts at compression sites occur after orthodontic appliance reactivation, when this is done during both the period of osteoclast recruitment and the peak expansion in the osteoclast population. This experiment examined osteoclasts and tooth movement in alveolar bone after appliance reactivation coinciding with alveolar bone formation and the time when reactivation osteoclasts first appear (ie, 10 days after initial appliance activation). Results showed that teeth in the reactivated group (Group I) displayed linear tooth movement (62.6 mm/day), and 0.9 mm tooth movement by day 10. Significant increases in osteoclast numbers, osteoclast surface percentage, and surface per individual osteoclast were evident in these animals by 1 day post reactivation ($P < .01$). These findings indicate that, after appliance reactivation during the time when reactivation osteoclasts appear, a second cohort of osteoclasts can be recruited immediately, along with

immediate and substantial tooth movement and no greater risk of root resorption.

Sappho Tzannetou, Stella Efstratiadis, (1998)[68] examined whether the inflammatory mediators interleukin (IL-1□) and □-glucuronidase (□G) are present in the gingival crevicular fluid (GCF) of children undergoing rapid palatal expansion and whether their levels vary upon activation of the appliance and movement of the maxillary first molars. The results indicate that (1) □G and IL-1□ are present in GCF of young, healthy individuals, (2) their levels decrease following a strict regimen of plaque control, (3) orthodontic/orthopedic forces evoke changes in the levels of the inflammatory mediators IL-1□ and □G in the periodontal tissues that can be detected in GCF. The results of this study support the hypothesis that mechanical stimulus causes an inflammatory reaction within the periodontal tissues, which in turn may trigger the biological processes associated with bone remodeling.

R.B.Johnson And F.G.Serio (2001)[59,31] did a study on leptin within healthy and diseased human gingiva. They

concluded that human leptin present within healthy and marginally inflamed gingiva and decreases in concentration as the adjacent probing depth increases. When leptin concentrations decreased vascular endothelial growth factor (VEGF) concentrations increased, suggesting that leptin could be released from gingiva coincident to vascular expansion. Thus gingiva in addition to adipose tissue could be a source of circulating leptin in patients with periodontal disease

Emanuela Serra, Giuseppe Perinetti, et al (2003)[19] examined the lactate dehydrogenase (LDH) activity in GCF to assess whether GCF LDH can be proposed as a sensitive marker for periodontal tissue modifications during orthodontic tooth movement. The results showed that no differences in clinical conditions and GCF volume occurred between the experimental teeth. On the contrary, GCF LDH activity in the test teeth was significantly greater than that of the control teeth (P _ .01). Moreover, no differences were found in the enzymatic activity between the sexes by experimental tooth, and no significant correlation was present between GCF LDH activity and patients' ages

within experimental teeth. Results indicated a possible role of GCF LDH during the early phases of orthodontic treatment

Kee-Joon Lee, Young-Chel Park, (2004)[36] did a study to evaluate the effects of a light continuous force and an interrupted force with weekly reactivation on interleukin-1_ (IL-1_) and prostaglandin E2 (PGE2); possible interactions between these 2 potent mediators of the bone resorption process were assessed in vivo. In each subject, 1 maxillary canine (E1) received continuous force with a nickel-titanium coil spring. The opposite canine (E2) received an interrupted force with a screw-attached retractor; An antagonistic canine was used as a control. The PGE2 level showed a significant elevation at 24 hours and then decreased. For E2, a significant elevation of IL-1_ level was observed at 24 hours and a greater significant elevation at 24 hours after the first reactivation, compared with the control sites. The PGE2 level increased significantly at 24 hours and remained high for 1 week. The synergistic up-regulation of PGE2 by appliance reactivation and secreted IL-1_ was not evident with either type of force after 1

week. Both experimental sites showed significant tooth movement compared with the control sites at 3 weeks; however, there was no significant difference between the 2 experimental sites. A well-controlled mechanical stress with timely reactivation cans effectively upregulate IL-1_ secretion.

Emel Sarı, Hu¨ seyin O¨ lmez, (2004)[20] conducted a study to examine the effects of 2 different anti-inflammatory drugs on gingival crevicular fluid (GCF) volume and on prostaglandin E2 (PGE2) levels of the GCF during orthodontic tooth movement. A total of 36 extraction patients, were divided into 3 groups. Acetylsalicylic acid (aspirin) and rofecoxib were used for pain control in the first and second groups; the third group was used as a control. Gingival crevicular fluid was sampled at the beginning of tooth movement and at 24, 48, and 168 hours. Depending on the variations of fibroblast activation, PGE2 levels of all the groups increased at 24 and 48 hours and decreased at 168 hours. When the drugs were compared, it was found that the inhibition effect of aspirin on PGE2 was more than that of rofecoxib. The results suggest that

rofecoxib can be used during orthodontic treatment, but further study is recommended.

Selin Kale, I˙ lken Kocadereli (2004)[71] compared the effects of local administrations of prostaglandin E2 (PGE2) and 1,25-dihydroxycholecalciferol (1,25-DHCC) on orthodontic tooth movement in rats.. There was no significant difference in tooth movement between the PGE2 and the 1,25-DHCC groups. Both PGE2 and 1, 25-DHCC enhanced the amount of tooth movement significantly when compared with the control group but , 1,25-DHCC was found to be more effective in modulating bone turnover during orthodontic tooth movement, because its effects on bone formation and bone resorption were well balanced.

Laura R. Iwasaki, Larry D. Crouch (2005)[40] **d**id a research to test 3 hypotheses: (1) the velocity of tooth translation (vt) is related to applied stress and growth status, (2) a threshold of stress accounts for the lag phase, and (3) vt is correlated with the ratio (AI) of 2 cytokines (IL-1_, IL-1RA) measured in gingival crevicular fluid (GCF) and stimulated whole blood (SWB). 0.78). It was

concluded that Vt varied with growth status and stresses _ 52 kPa; stresses of _ 52 kPa showed no lag phase; and equivalent stresses yielded subject-dependent differences in vt, which correlated with cytokines in GCF .

Vinod Krishnan and Ze'ev Davidovitch (2006)[84] stated that remodeling changes in paradental tissues are considered essential in effecting orthodontic tooth movement. The force-induced tissue strain produces local alterations in vascularity, as well as cellular and extracellular matrix reorganization, leading to the synthesis and release of various neurotransmitters, cytokines, growth factors, colony-stimulating factors, and metabolites of arachidonic acid. Their review aims to achieve this goal and is organized to include all major findings from the beginning of research in the biology of tooth movement. It highlights recent developments in cellular, molecular, tissue, and genetic reactions in response to orthodontic force application. It reviews briefly the processes of bone, periodontal ligament, and gingival remodeling in response to orthodontic force. This review also provides insight into

the biological background of various deleterious effects of orthodontic forces.

Richard S. Masellaa and Malcolm Meisterb (2006)[64] stated that five micro-environments are altered by orthodontic force: extracellular matrix, cell membrane, cytoskeleton, nuclear protein matrix, and genome. Gene activation (or suppression) is the point at which input becomes output, and further changes occur in all 5 environments. Gene-directed protein synthesis, modification, and integration form the essence of all life processes, including OTM. Cell membrane receptor-ligand docking is an important initiator of signal transduction and a discovery target for new bone-enhancing drugs. Interpatient variation in mechanobiological response is most likely due to differences in periodontal ligament and bone cell populations, genomes, and protein expression patterns. Discovery of mutations in OTM-associated genes of orthodontic patients, including those regulating osteoclast bone-matrix acidification, chloride channel function, and osteoblast-derived mineral and protein matrices, will permit gene therapy to restore normal matrix

and protein synthesis and function. Achieving selectivity in targeting abnormal genes, cells, and tissues is a major obstacle to safe and effective clinical application of gene engineering and stem-cell mediated tissue growth.

Melih Y. Sueri, Tamer Turk (2006)[48] did a study to evaluate the effects of lace back ligatures on canine distalisation during the leveling and aligning stage and to compare the effectiveness of lace backs ligatures with that of super elastic NiTi coil springs. For canine distalization super elastic NiTi coil springs generating 150 gm of force were use on one side. Lace backs made from 0.010 inch ligature wires were applied on contralateral side. Results showed that canine and molar movements were greater for the coil group than for the laceback group and the differences were significant.

Oscar R. Ariasa and Maria C. Marquez-Orozcob (2006)[56] conducted a study to determine by direct measurement the effects that acetylsalicylic acid, ibuprofen, and acetaminophen have on orthodontic tooth movement in rats and to evaluate histologically the differences in bone

resorption in the pressure area in rats treated with these analgesics. There was no significant difference between the acetaminophen group and the control group, or between the aspirin and ibuprofen groups. Tooth movement was similar in the groups. The results indicate that nonsteroidal anti-inflammatory analgesics such as aspirin and ibuprofen diminish the number of osteoclasts, probably by inhibiting the secretion of prostaglandins, thereby reducing orthodontic tooth movement. Acetaminophen did not affect orthodontic tooth movement in rats, and it might be the analgesic of choice for treating pain associated with orthodontic treatment.

Giuseppina Cantarella, Rosita Cantarella, (2006)[26] did a. investigation to evaluate matrix metalloproteinase (MMP)-1 and MMP-2 in the GCF of human teeth exposed to orthodontic force on both the tension and compression sides in the initial phase of orthodontic tooth movement. Orthodontic force was applied by using a Sentalloy coil-spring) of 150 g. The GCF sampling on the mesiobuccal and distobuccal aspects of each experimental and control tooth was performed at specific times up to 8 hours with paper

strips. Results showed that compression force induced a significant increase of MMP-1 protein after 1 hour; the increase lasted until the third hour of force application and disappeared thereafter. The tension force induced significantly increased levels of the MMP-1 protein after just 1 hour of force application. MMP-2 protein was induced by compression and increased significantly in a time-dependent fashion, reaching a peak after 8 hours of force application. On the tension side, MMP-2 was significantly increased after 1 hour but gradually returned to basal levels within 8 hours. It was concluded that Orthodontic forces affect both MMP-1 and MMP-2 protein levels on the compression and the tension sides, although to different extents, whereas MMP-1 and MMP-2 protein levels change in a time-dependent fashion.

Yesim.Bozkurt Et Al (2006)[90] did a study on leptin levels in GCF in periodontitis patients with long term and heavy smoking .They concluded that higher leptin GCF levels in healthy sites in periodontitis patients may play a protective role in periodontal disease.

Güvenç Bas¸ aran, Törün Özer,et al (2006)[28] did a study in which the aims were to determine levels of interleukins 2, 6, and 8 during tooth movement, and test whether they differ from each other with leveling and distalization forces used in various treatment stages of standard orthodontic therapy. Results showed that increases were seen in the volume of gingival crevicular fluid and the concentrations of interleukins 2, 6, and 8. Hence it was concluded that Leveling and distalization of the teeth evoke increases in interleukins 2, 6, and 8 levels in the periodontal tissues that can be detected in gingival crevicular fluid

Karthikeyan, B. V. and Pradeep, A. R. (2007)[34] concluded that as periodontal tissue destruction increased, there was a substantial decrease in gingival crevicular fluid leptin concentration. This observation extends our knowledge of the protective role of leptin in periodontal health.

Levent Kardeşler , Nurcan Buduneli (2008)[41] did a study to evaluate if type 2 diabetes mellitus increase gingival crevicular fluid (GCF) levels of prostaglandin E2 (PGE2), interleukin- 1beta (IL-1ß), tissue-type plasminogen

activator (t-PA), and plasminogen activator inhibitor-2 (PAI-2). Results showed that DM group revealed lower IL-1ß levels than PD group. PGE2, t-PA and PAI-2 levels were similar in DM and PD groups. PGE2, t-PA levels were higher in DM and PD groups than H group. PAI-2 level was higher in DM group than H group. GCF total amount of PGE2 in DM group exhibited significant correlations with all clinical periodontal measurements. It was concluded that Type 2 diabetes in this study seems not to increase GCF levels of the evaluated inflammatory mediators 2

Masako Yoshimatsu, Masataka Uehara (2008)[46] stated that Heat shock protein 47 (HSP47) is a molecular chaperone specifically involved in the processing and quality control of collagen molecules. HSP47 is expressed in the endoplasmic reticulum of cells producing type I collagen and in the intercellular collagenous matrices, and it is actively involved in type I collagen biosynthesis. It was therefore considered of value to investigate HSP47 expression in the PDL during tooth movement. The aim of his study was to investigate the kinetics of heat shock protein 47 (HSP47) and proliferating cell nuclear antigen

(PCNA) immunohistochemistry in periodontal ligament (PDL) cells during orthodontic tooth movement in a mouse model. HSP47 expression was significantly higher on the tension side 2 days after application of the appliance, whereas no significant change was observed on the pressure side at any time point. Furthermore, the PCNA labelling indices of PDL cells were increased significantly on the tension side 6 and 10 days after application of the appliance, and on the pressure side 2, 6 and 10 days after application of the appliance. These data suggest that collagen is metabolized predominantly on the tension side, and that PDL cells actively proliferate on both the tension and pressure sides during orthodontic tooth movement. l

Theodosia Bartzela, Jens C. Türp, Edith Motschall (2009)[78] published a systematic literature review on the effects of medications and dietary supplements on the rate of experimental tooth movement. Forty-nine articles were included in the review, but their interpretation was hindered by the variability in experimental design, magnitude of force applied during tooth movement, and medication regimens. Therapeutic administration of eicosanoids

resulted in increased tooth movement, whereas their blocking led to a decrease. Nonsteroidal anti-inflammatory drugs (NSAIDs) decreased tooth movement, but non-NSAID analgesics, such as paracetamol (acetaminophen), had no effect. Corticosteroid hormones, parathyroid hormone, and thyroxin have all been shown to increase tooth movement. Estrogens probably reduce tooth movement, although no direct evidence is available. Vitamin D3 stimulates tooth movement, and dietary calcium seemed to reduce it. Bisphosphonates had a strong inhibitory effect. It was concluded that medications might have an important influence on the rate of tooth movement, and information on their consumption is essential to adequately discuss treatment planning with patients.

Patricia Joyce Brooksa; Dorrin Nilforoushanb (2009)[57] conducted a study to understand the molecular basis of early orthodontic tooth movement by looking at the expression of KI-67, runt-related transcription factor 2 (Runx2), and tumor necrosis factor ligand superfamily member 11 (RANKL) proteins. Results showed increased expression of KI-67, a proliferation marker, and RANKL, a

molecule associated with osteoclastic differentiation, in the compression sites of the periodontal ligament subjected to 3 hours of force. In contrast, there was increased expression of KI-67 and Runx2, a marker of osteoblast precursors, in tension areas after 24 hours of force. Decreased KI-67 expression in the mesial and distal regions of the periodontal ligament was observed at the midpoint of the tooth root. Thereby it was concluded that the early RANKL expression indicates that at this early stage cells are involved in osteoclast precursor signaling. Also, decreased KI-67 expression found near the midpoint of the tooth root is believed to represent the center of rotation, providing a molecular means of visualizing mechanical loading patterns

Yamaguchi, M. (2009)[88,35] found that concentrations of RANKL in GCF is increased during orthodontic tooth movement, and the ratio of concentration of RANKL to that of OPG in the GCF. *In vivo* studies have shown the presence of RANKL and RANK in periodontal tissues during experimental tooth movement of rat molars, and that PDL cells under mechanical stress may induce osteoclastogenesis through upregulation of RANKL

expression during orthodontic tooth movement. Hence it is concluded that the RANKL, and OPG are important in physiologic osteoclast formation, it is reasonable to propose that the RANKL/RANK/OPG system plays an important role in orthodontic tooth movement

Andrea M. Marcaccini, Patricia A.F. Amato, Fernanda (2010)[5] **c**onducted a study in which the aim was to determine MPO activity in the GCF and saliva (whole stimulated saliva) of orthodontic patients at different time points after fixed appliance activation. GCF and saliva samples were collected at baseline, 2 hours, and 7 and 14 days after application of the orthodontic force. Results showed mean MPO activity was increased in both the GCF and saliva of orthodontic patients at 2 hours after appliance activation. At 2 hours, PMN infiltration into the periodontal ligament from the orthodontic force probably results in the increased MPO level observed at this time point. Hence it was concluded that MPO might be a good marker to assess inflammation in orthodontic movement; it deserves further studies in orthodontic therapy.

Andrea Wichelhaus, Lorenz Brauchli, (2010)[6] stated that the main advantage of superelastic nickel-titanium (NiTi) products is their unique characteristic of force plateaus, which allow for clinically precise control of the force. The aims of their study were to define the mechanical characteristics of several currently available closed-coil retraction springs and to compare these products. It was concluded that in sliding mechanics, the strongly superelastic closed-coil springs with preactivation are recommended. In addition, it was found that the oral environment seems to have only a minor influence on their mechanical properties.

STUDIES RELATED TO LEPTIN

Piotr C. Konturekb, Stanislaw J. Kontureka (2001)[58] have stated that Leptin, encoded by the ob gene, is known mainly for its role in the regulation of food intake, body composition and energy expenditure through a central feedback mechanism. Initially leptin was considered as an ob gene product of adipocytes but recently the presence of leptin and its receptors have been revealed in other organs including gastric mucosa and the pancreas and found to be released from these organs by cholecystokinin (CCK), gastrin and ordinary feeding. Furthermore, leptin was found to mimic the action of CCK on gastric and pancreatic integrity, while reducing the food intake and to affect gastric and pancreatic secretion. This report emphasizes the role of leptin originating from the gastrointestinal tract acting synergistically with CCK at the hypothalamus level on the mechanism of food intake and locally on the protection of gastric mucosa and the pancreas against noxious agents and to maintain tissue integrity.

M. Tena-Sempere (2002)[45] has stated strongly suggested that leptin is able to act at different levels of the

hypothalamic-pituitary-testicular axis. Leptin appears to act as a direct inhibitory signal for testicular steroidogenesis, which may be relevant to explain the link between decreased testosterone secretion and hyperleptinaemia in obese men. Analysis of the molecular basis for leptin-induced inhibition of testosterone secretion revealed the potential involvement of decreased gene expression of several up-stream factors in the steroidogenic pathway. Overall, data indicate that the testis is a direct target for leptin actions. Furthermore, the available evidence is suggestive of a tightly regulated, complex mode of action of leptin at different levels of the male gonadal axis that involves not only stimulatory but also inhibitory effects

Jean L. Chan et al (2002) stated that even after correcting for body weight and fat mass, women have higher serum leptin levels than men. This sexual dimorphism in serum leptin concentrations has been associated with or is causally related to a number of factors. First, the pulse amplitude, but not the pulse frequency, of leptin secretion from adipose tissue is twofold to threefold higher in females than in males. Second, fat mass is increased in females, and

there is differential fat distribution with a higher subcutaneous/visceral fat ratio in women than men. Leptin mRNA expression is known to be higher in subcutaneous than visceral fat depots .Third, women have higher total serum leptin levels but lower leptin-binding protein levels than men, indicating higher free leptin levels Finally, female adipose tissue may be more sensitive to hormones (i.e., insulin and glucocorticoids) or other substances that stimulate leptin production. It is known that sex steroids such as estrogens increase leptin levels whereas androgens decrease leptin levels

Darleen A. Sandoval, Stephen N. Davis (2003)[16] did a study in which the purpose was to critically analyze the literature regarding the impact of different types of stress on leptin secretion, the function of leptin during stress, and the role of leptin in the pathophysiology of diabetes. While it is clearly evident that leptin is decreased during caloric restriction, the response of leptin to other types of stress has been plagued by conflicting data. With hypoglycemia stress, the literature may conflict because experimentally hypoglycemia is induced with infusion of insulin, an

endocrine factor that can increase leptin levels. With exercise, leptin's response may depend on duration and intensity of exercise. While it has been clearly shown that the sympathetic nervous system (SNS) inhibits leptin secretion in a variety of experimental modes, the hypothalamic–pituitary–adrenal (HPA) axis may stimulate leptin secretion. This creates a paradox of leptin regulation during stress since both systems are activated with stress. In type 1 diabetes mellitus, autonomic dysfunction may prevent the fall in leptin during stress. Although obesity is associated with type 2 diabetes mellitus, patients may have decreased leptin levels, especially when glucose is poorly controlled. This may contribute to further obesity and worsening of the disease.

Berna Binnur Kıvırcıka,, Ko¨ksal Alptekina (2003)[10], did a study in which the aim was to investigate the influence of clozapine on hormones leptin and insulin in relation to body weight and composition measures to determine their contribution to clozapine-induced weight gain. The results showed that Leptin and insulin levels did not show any significant alterations across time. The use of

clozapine was associated with significant increases in BMI significantly decreased. . The change in leptin levels was correlated to change in body fat mass. It was concluded that the role of leptin in weight gain induced by clozapine might be a regulatory mechanism rather than being etiologic

Julie A. Meyers, Anne McTiernana (2005)[32] in their review presented a summary of published research relating serum leptin concentrations to measures of inflammation and immune function. In vitro and animal studies suggest a multifunctional role of leptin in immune function, including associations with the proinflammatory TH1 response, natural killer cell cytotoxicity, C-reactive protein, IL-6, tumor necrosis factor a, and, possibly, with serum amyloid A. It is difficult to discern whether there are also direct effects of cytokines on leptin; yet, at least with respect to tumor necrosis factor a, some studies suggest such a link.

Susan A. Farr William A. Banks et al (2005)[77] have stated that Leptin also acts in the hippocampus where it facilitates the induction of long-term potentiation and enhances NMDA receptor-mediated transmission. This suggests that

leptin plays a role in learning and memory. Obese mice and rats, which have leptin receptor deficiency, have impaired spatial learning. In disease states such as diabetes, humans and animals develop leptin resistance at the BBB. This suggests that low leptin levels in the brain may be involved in cognitive deficits associated with diabetes. Their results indicated that leptin in the hippocampus is involved in memory processing and suggests that low levels of leptin may be involved in cognitive deficits seen in disease states where leptin transport into the CNS is compromised.

Seth S. Martin, , Atif Qasim, , Muredach P. Reilly (2008)[72], have discussed about **i**ncreased circulating leptin, a marker of leptin resistance, that is common in obesity and independently associated with insulin resistance and cardiovascular disease (CVD) in humans. The mechanisms of leptin resistance include genetic mutation, leptin self-regulation, limited tissue access, and cellular or circulating molecular regulation. Evidence suggests that central leptin resistance causes obesity and that obesity-induced leptin resistance injures numerous peripheral tissues, including liver, pancreas, platelets, vasculature, and myocardium.

This metabolic- and inflammatory-mediated injury may result from either resistance to leptin's action in selective tissues, or excess leptin action from adiposity associated hyperleptinemia. In this sense, the term "leptin resistance" encompasses a complex pathophysiological phenomenon. Leptin is even purported to physically interact with C-reactive protein, resulting in leptin resistance, which is particularly intriguing, given C-reactive protein's well-studied relationship to cardiovascular disease. Given that plasma levels of leptin and inflammatory markers are correlated and also predict cardiovascular risk, it is conceivable that part of this risk may be mediated through leptin resistance-related insulin resistance, chronic inflammation, type II diabetes, hypertension, atherothrombosis, and myocardial injury. Leptin resistance and its interactions with metabolic and inflammatory factors, therefore, represent potential novel diagnostic and therapeutic targets in obesity-related cardiovascular disease.

Rocı´o Lago, Rodolfo Go´mez a, Francisca Lago (2008)[65] have stated that Leptin, a 16 kDa non-glycosylated

polypeptide produced primarily by adipocytes and released into the systemic circulation, exerts a multitude of regulatory functions including energy utilization and storage, regulation of various endocrine axes, bone metabolism, and thermoregulation. In addition to leptin's best known role as regulator of energy homeostasis, several studies indicate that leptin plays a pivotal role in immune and inflammatory response. Because of its dual nature as a hormone and cytokine, leptin can be nowadays considered the link between neuroendocrine and immune system. The increase in leptin production that occurs during infections and inflammatory processes strongly suggests that this adipokine is a part of the cytokines network which governs Inflammatory/immune response and host defence mechanisms. Indeed, leptin plays a relevant role in inflammatory processes involving either innate or adaptive immune responses.

Chung-Hua Hsu, Su-Ching Lin, Kung-Chang Hwang (2008)[14] did a study in which the aim was to examine the gender differences in leptin level in a homogeneous Type 2 diabetic cohort and the factors contributing to such a

difference. Results of the study demonstrated that Type 2 diabetic women had higher plasma leptin concentrations than their male counterparts ($p < 0.001$). It was concluded that men had lower leptin levels than women, and seem to indicate that insulin concentration is the main predictor of leptin level in both Type 2 diabetic men and women.

Giamila Fantuzzi (2008) [25] has discussed 3 issues namely (1) Where am I (leptin) going, or what is the cellular target of leptin for modulation of immune responses? (2) Where am I coming from, or Is the cellular source important in determining leptin's effects on immune responses? and (3) What am I doing, or What are leptin's effects on immune and inflammatory responses?

Satya P. Kalra (2008)[70] in his review has stated about the recent scientific evidence concerning central leptin insufficiency versus leptin resistance formulations to explain metabolic and neural disorders resulting from subnormal or defective leptin signaling in various sites in the brain. The cumulative new knowledge favors a unified central leptin insufficiency syndrome over the, in vogue,

central resistance hypothesis to explain the global adverse impact of deficient leptin signaling in the brain. Furthermore, the leptin insufficiency syndrome delineates a novel role of leptin in the hypothalamus in restraining rhythmic pancreatic insulin secretion while concomitantly enhancing glucose metabolism and non-shivering thermogenic energy expenditure, sequelae that would otherwise promote fat accrual to store excess energy resulting from consumption of energy-enriched diets.

Tina A. Dardeno , Sharon H. Chou et al (2010) [81] in their review have stated that the role of leptin in human physiology and review evidence from recent ‘‘proof of concept” clinical trials using recombinant human leptin in subjects with congenital leptin deficiency, hypoleptinemia associated with energy-deficient states, and hyperleptinemia associated with garden-variety obesity. Since most obese individuals are largely leptin-tolerant or -resistant, therapeutic uses of leptin are currently limited to patients with complete or partial leptin deficiency, including hypothalamic amenorrhea and lipoatrophy. Leptin administration in these energy-deficient states may help

restore associated neuroendocrine, metabolic, and immune function and bone metabolism. Leptin treatment is currently available for individuals with congenital leptin deficiency and congenital lipoatrophy. The long-term efficacy and safety of leptin treatment in hypothalamic amenorrhea and acquired lipoatrophy are currently under investigation. Whether combination therapy with leptin and potential leptin sensitizers will prove effective in the treatment of garden-variety obesity and whether leptin may have a role in weight loss maintenance is being greatly anticipated.

MATERIALS AND METHODS

25 orthodontic subjects including 13 boys and 12 girls in the age group of 16- 20 years attending the outpatient Department of Orthodontics and Dentofacial Orthopedics Of Tamilnadu Government Dental College & Hospital Chennai constitute the sample. Patients rights were protected, Comprehensive procedural information was given to all patients and written informed consent obtained. Ethical clearance was obtained from the Institutional Ethical Committee of Tamilnadu Govt. Dental College & Hospital, Chennai.

Inclusion criteria

Subjects those who fulfilled the following criteria were only included in the study:

- Orthodontic patients requiring maxillary 1st PM extraction and distal movement of canines
- Good health
- Normal body mass index

- No use of anti-inflammatory drugs within the month preceding the study
- No history of antimicrobial therapy within previous 6 months
- Healthy periodontal tissues with generalized probing depth of less than or equal to 2 mm with minimal bleeding
- No history of chronic medication that may have effect on leptin levels (oral contraceptives and antipsychotics)[98]
- No radiographic evidence of periodontal bone loss
- Patients who have signed the informed consent

Oral prophylaxis was done for all subjects following which oral hygiene instructions were given before placement of orthodontic appliances. To avoid leptin derived from obese subjects biasing the estimation of leptin concentration, these subjects were excluded from the study by selecting only subjects with a normal body mass index (18.5–22.9 kg/m^2) according to a chart for the Asian population given by the World Health Organization in 2002.

To rule out any drug effects on leptin concentration proper medical history is elicited.

EXPERIMENTAL DESIGN

Before placement of orthodontic appliance, gingival crevicular fluid samples were collected from all subjects from left maxillary canines. Then maxillary left and right 1st premolar extractions were done. Fixed Orthodontic appliances were placed 1 week following extractions. Orthodontic brackets (0.022 slot 3M Roth) were placed in both arches. Upper triple molar tube and lower double molar tubes were used. After leveling the maxillary arch, left side canine was retracted with 9mm size (0.012 x 0.030 inch) NiTi coil spring along 17 x 25 SS wire. The spring delivers a light constant continuous force of 150-200 gm. The contra lateral canine ie. the right maxillary canine did not receive any distal retractive force.

PERIODONTAL EXAMINATION

Orthopanomogram was taken for each subject to rule of any radiographic evidence of generalized periodontal bone loss. Intraoral periapical radiographs were taken for

right and left maxillary canines to specifically rule out any periodontal bone loss around these teeth that undergo significant distal movement. For each subjects, plaque index and gingival bleeding index were recorded within 15 seconds after probing. Probing depth scores were also recorded. All these clinical parameters were assessed twice; at the baseline and at the end of the study. All clinical data were collected by the same investigator.

GINGIVAL FLUID COLLECTION

All the GCF samples were collected around 10 am. GCF collection was performed before periodontal probing to avoid mechanical irritation or bleeding by penetration of probe. Supra gingival plaque if present at the time of sampling was removed. The teeth were gently dried with air spray and isolated with cotton rolls. Retraction of cheeks was done with cheek retractor. Salivary ejector was used to avoid salivary contamination.

GCF was collected using gingival fluid collection strips (Perio paper). The first strip was inserted into the disto buccal crevice of maxillary right canine to a level

1mm below the gingival margin and held in place for 30 seconds. After 1 minute the second strip was inserted into the distopalatal crevice and held in place for 30 seconds. Extra care was taken to avoid blood and saliva contamination. Strips contaminated with blood or saliva were discarded.

GCF volume measurement

The volume of GCF collected in the strips were measured by the chair side electronic gingival fluid measuring device **(Periotron)** which was calibrated using known volumes of phosphate buffered saline.

TIMING OF THE SAMPLE

Totally 5 GCF samples are collected from each subject Pretreatment – from disto buccal and disto palatal crevice of right side maxillary canine. (S**ample A.)**

After maxillary arch is aligned upto 17 x25 SS wire stage, retractive force is applied with 9 size NiTi coil spring to the maxillary left side canine and not to the right side canine. 6 hours after applying this distal retractive force

GCF is collected from both maxillary right **(Sample B)** and maxillary left canine **(Sample C).**

After 21 days GCF is again collected from the maxillary right **(Sample D)** and maxillary left (**Sample E)** canines.

Previous studies did not compare the leptin levels around an obsolutely stable tooth and tooth under orthodontic movement. So, in this study, pretreatment leptin levels, ie. leptin levels around the tooth when it is completely stable is also measured.

4- 6 hours is the critical time period during tooth movement when second messengers are released that are very important for cellular functions including differentiation

21st day is the one in which the appliance is usually reactivated after giving the periodontal tissues a time period for repair and regeneration. [91].

STORAGE

After the measurement of GCF volume, the strips were transferred to Eppendorf tubes (Micro centrifuge tubes) and isolated with Parafilm to avoid evaporation. The Eppendorf tubes were subjected to physical agitation in cyclotron to make the solid particles settle down. Then each sample was labeled and stored at -80^{o}c (Deep freezer) until the assay was performed.

LEPTIN ANALYSIS

Each strip was eluted twice with 100 microlitres of Hanks Balanced Salt Solution containing 0.5% Bovine Serum albumin by centrifugation (3000 x g; 4^{o}c) for 15 minutes. Leptin concentration was measured by commercially available enzyme linked immunosorbent assay. The assay was conducted according to the manufacturer's instructions. For leptin assays high sensitive kits were used to quantitatively detect low levels of leptin which was bound to antileptin, monoclonal coating antibody absorbed by the microwells. The second polyclonal antibodies were added and after incubation coloured products were formed in proportion to the amount of leptin

present in the sample. The reactions were measured at 450 nm. The total leptin was determined in picograms (pg). The calculation of concentration in each sample was performed by dividing the amount of leptin by the volume of the sample (pg/ microlitre).

HANKS BALANCED SALT SOLUTION

It is a Buffer solution and is used to prevent the degradation of body fluids (GCF) outside the body temperature

Composition

- NaCl - 397mg / 50ml
- KCl - 20mg / 50ml
- NaHPO4 - 4mg / 50ml
- KH2PO4 - 3mg / 50ml
- NaHCO3 -18mg / 50ml
- Bovine albumin serum – 0.05%

PHOTO PLATE:1

Fig 1; Probing depth measurement

Fig 2: Indexing

PHOTO PLATE: 2

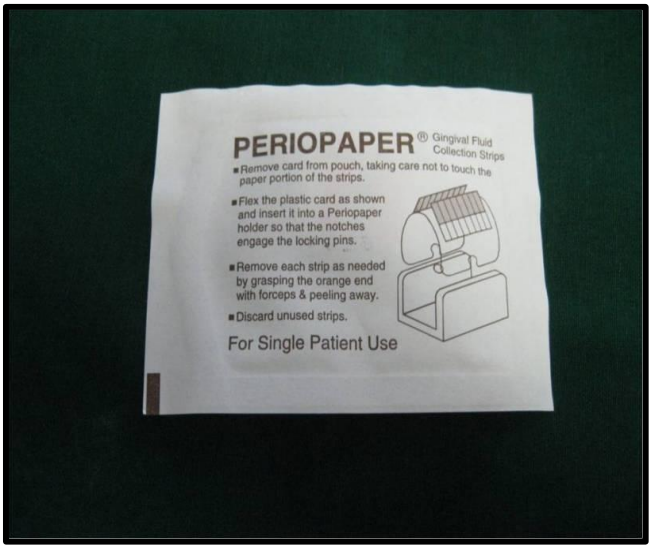


Fig 3:GCF collection strips

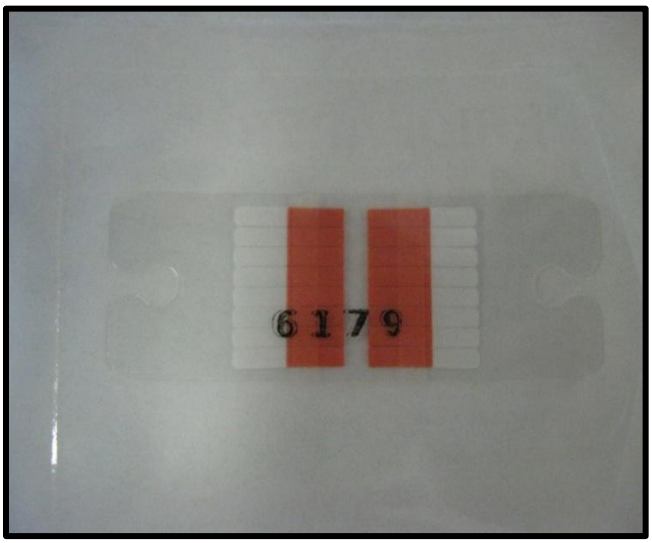


Fig 4: Periopaper

PHOTO PLATE: 3

Fig : 5 Pretreatment

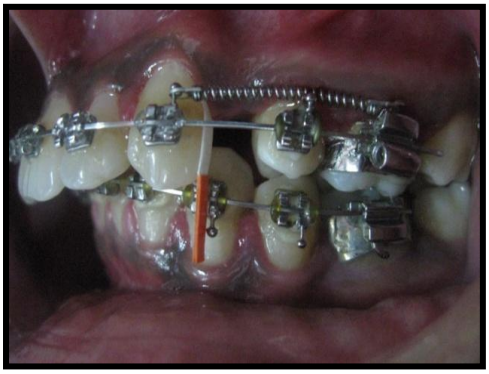


Fig 6: Test tooth

Fig : 7 Control tooth

PHOTO PLATE: 4

Fig:8 Periotron

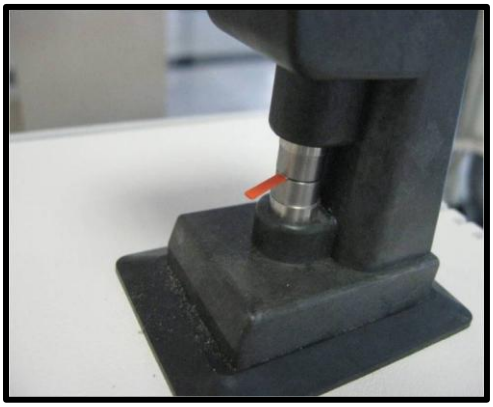


Fig: 9 GCF strips in Periotron

Fig : 10 GCF volume measurement in periotron

PHOTO PLATE: 5

Fig 11: HBSS

Fig 12: Transferring HBSS into Microcentrifuge Tubes

Fig 13: Labelling

PHOTO PLATE: 6

Fig 14: Cyclotron

Fig 15: Centrifuge

PHOTO PLATE : 7

Fig 16: Deep Freezer

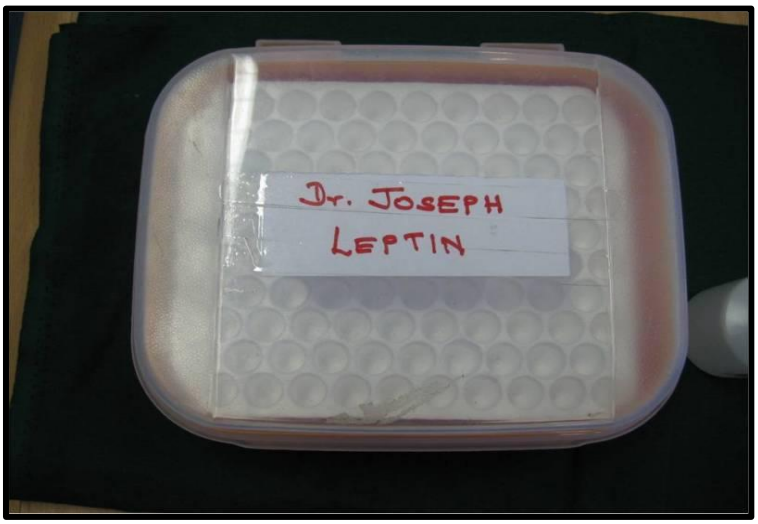


Fig 17: Storage Box

PHOTO PLATE: 8 leptin kit

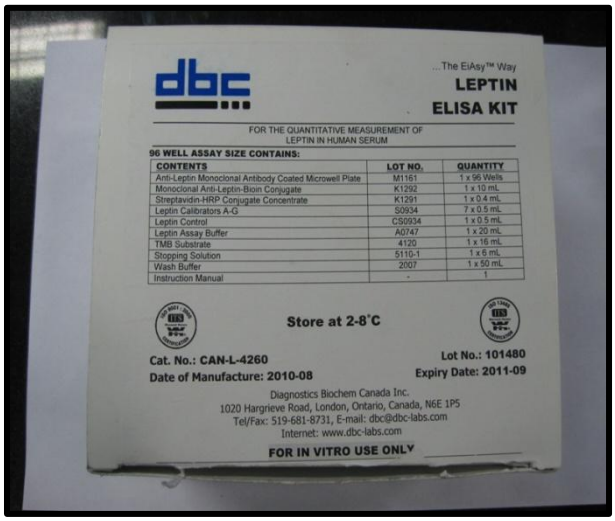


Fig 18,19,20 : leptin kit

PHOTO PLATE: 9

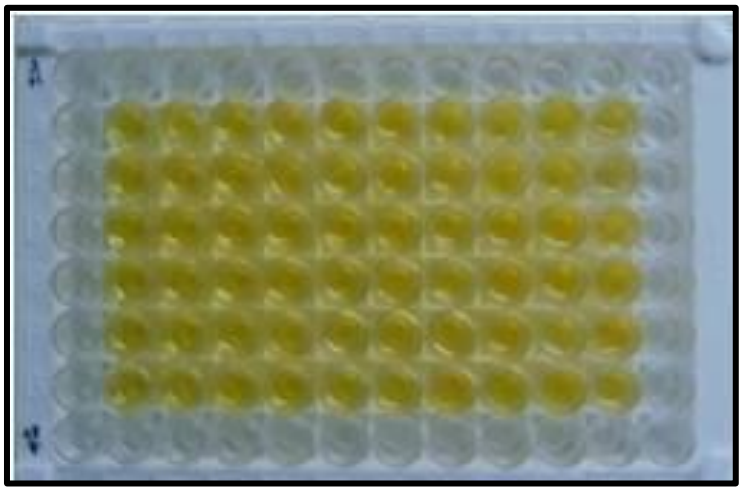


Fig 21; Leptin Detection By Formation of Coloured Products

Fig 22: ELIZA Reader

RESULTS

As oral hygiene instructions were severely given to all patients, plaque accumulation was minimal throughout the study period. There was no bleeding on probing or loss of attachment. Probing depth remained less than 2mm throughout the study period.

GCF values:

- The amount of GCF in pretreatment, 6 hour control tooth, 21st day control and test tooth were all **similar.**
- The amount of GCF in 6 hour test tooth was **elevated** that was statistically significant.(One way Anova test: $P < 0.01$) (Tukey HSD test : $P < 0.05$)
- GCF volumes of all the 25 subjects at pretreatment (A), 6 hour test tooth site (B), 6 hour control tooth site(C), 21st day test tooth site (D), 21st day control tooth site (E) are shown in **table 1**

Leptin values:

- GCF Leptin concentration in pre treatment, 6 hour control and 21st day control were all similar.
- GCF leptin concentration at 6 hour test tooth site was increased which was statistically significant. .(One way Anova test: P <0.01) (Tukey HSD test: P < 0.05)
- GCF leptin concentration in 21st day test tooth was decreased only a little than the control tooth which shows statistically no difference. (P> 0.05)
- Moreover as an additional finding, the leptin concentration of all the girls were higher than the boys even after correcting for body mass.
- The GCF leptin concentration of all the 25 subjects at pretreatment (A),6 hour test site (B), 6 hour control site(C), 21st day test site (D), and 21st day control site (E) are shown in **table 2**

Table : 1 GCF volume (µL) in all subjects

S.No	A(pretreatment)	B(6 hr test tooth)	C(6 hr control tooth)	D(21st day test tooth)	E(21st day control tooth)
1.	0.76	0.83	0.81	0.72	0.75
2.	0.71	0.89	0.83	0.69	0.63
3.	0.52	0.92	0.72	0.58	0.59
4.	0.83	0.96	0.69	0.81	0.8
5.	0.81	0.98	0.76	0.72	0.72
6.	0.82	0.87	0.79	0.78	0.78
7.	0.76	0.76	0.75	0.74	0.74
8.	0.79	0.91	0.81	0.73	0.81
9.	0.59	0.73	0.63	0.62	0.69
10.	0.72	0.72	0.62	0.7	0.7
11.	0.74	0.8	0.7	0.74	0.7
12.	0.69	0.81	0.71	0.72	0.69
13.	0.76	0.81	0.69	0.67	0.74
14.	0.88	0.91	0.85	0.82	0.81
15.	0.67	0.96	0.87	0.8	0.63
16.	0.72	0.99	0.68	0.69	0.69
17.	0.76	0.82	0.76	0.7	0.7
18.	0.81	1.09	0.78	0.74	0.75
19.	0.83	0.89	0.79	0.75	0.78
20.	0.69	0.86	0.77	0.71	0.62
21.	0.72	0.95	0.89	0.82	0.71
22.	0.81	1.01	0.81	0.8	0.8
23.	0.83	0.87	0.79	0.73	0.79
24.	0.79	0.9	0.82	0.8	0.75
25.	0.64	0.74	0.68	0.69	0.65

Table: 2 Leptin levels in GCF (pg/µl) in all subjects

S.No	A(pretreatment)	B(6 hr test tooth)	C(6 hr control tooth)	D21st day test tooth)	E(21st day control tooth)
1.	0.75	0.88	0.72	0.7	0.77
2.	0.74	0.87	0.74	0.71	0.75
3.	0.74	0.88	0.73	0.69	0.74
4.	0.8	0.88	0.81	0.78	0.81
5.	0.7	0.84	0.77	0.66	0.72
6.	0.72	0.89	0.72	0.68	0.72
7.	0.68	0.77	0.69	0.64	0.67
8.	0.6	0.72	0.62	0.6	0.59
9.	0.88	0.98	0.82	0.82	0.88
10.	0.78	0.88	0.78	0.75	0.77
11.	0.8	0.92	0.82	0.77	0.81
12.	0.86	0.95	0.87	0.8	0.85
13.	0.72	0.85	0.75	0.64	0.72
14.	1.19	1.1	0.99	1.01	1
15.	0.66	0.78	0.68	0.61	0.65
16.	0.74	0.87	0.71	0.7	0.72
17.	0.74	0.88	0.75	0.7	0.73
18.	0.8	0.93	0.79	0.74	0.79
19.	0.8	0.92	0.74	0.75	0.78
20.	0.7	0.81	0.68	0.66	0.7
21.	0.64	0.74	0.69	0.6	0.63
22.	0.62	0.7	0.65	0.57	0.63
23.	0.8	0.89	0.83	0.76	0.81
24.	0.72	0.83	0.73	0.65	0.73
25.	0.6	0.75	0.67	0.54	0.59

Table 3: Mean and Standard Deviation of GCF volume (µ l) In Test Tooth And Control Tooth Throughout The Study Period

	Pre treatment	**6 hour**	**21st day**	**P value**
Control tooth	0.74+ .082	0.76+.07	0.72+.06	**P<0.001**
Test tooth		0.87+.09	0.73+.05	

Table : 4 Mean and Standard Deviations Of Levels Of GCF Leptin (pg/µl) In The Test Tooth And Control Tooth Throughout The Study Period

	Pretreatment	**6 hr**	**21st day**	**P value**
Control tooth	**0.75+.11**	**0.75+.07**	**0.74+.09**	**P<0.001**
Test tooth		**0.86+.08**	**0.70+.09**	

CHART - 1

Gingival Crevicular Fluid Volume (µ l) During Orthodontic Treatment

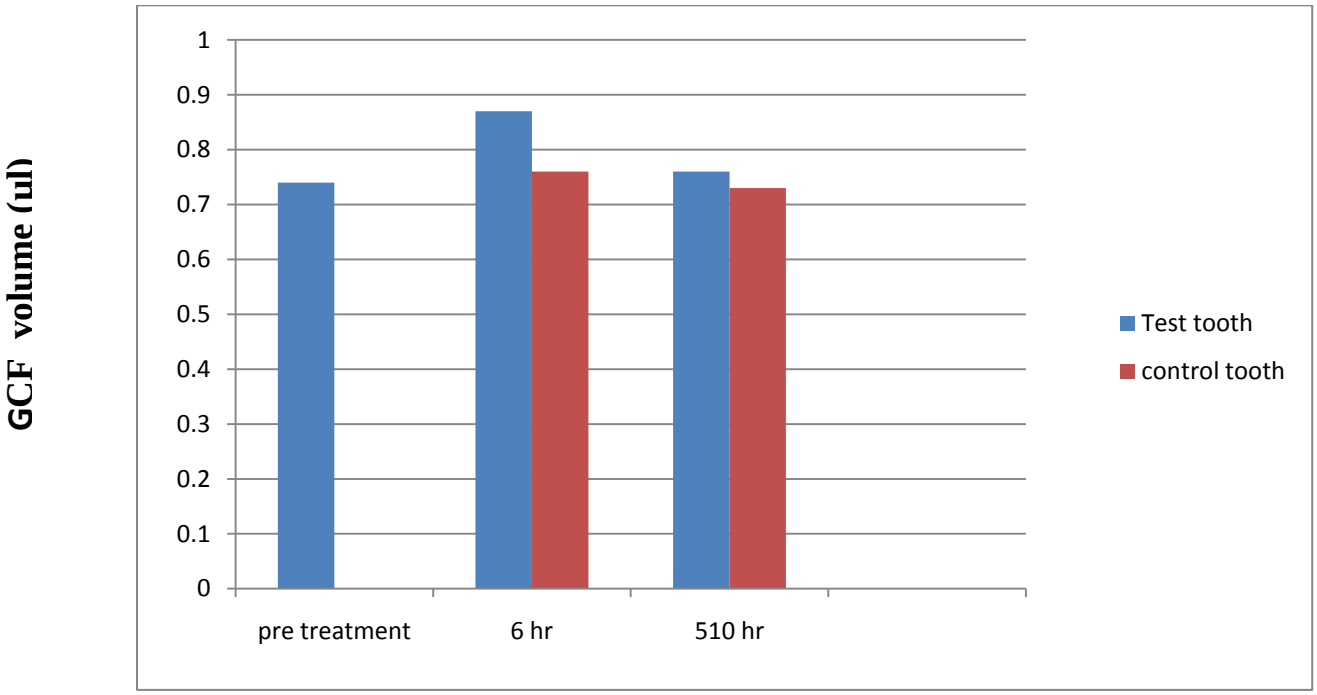
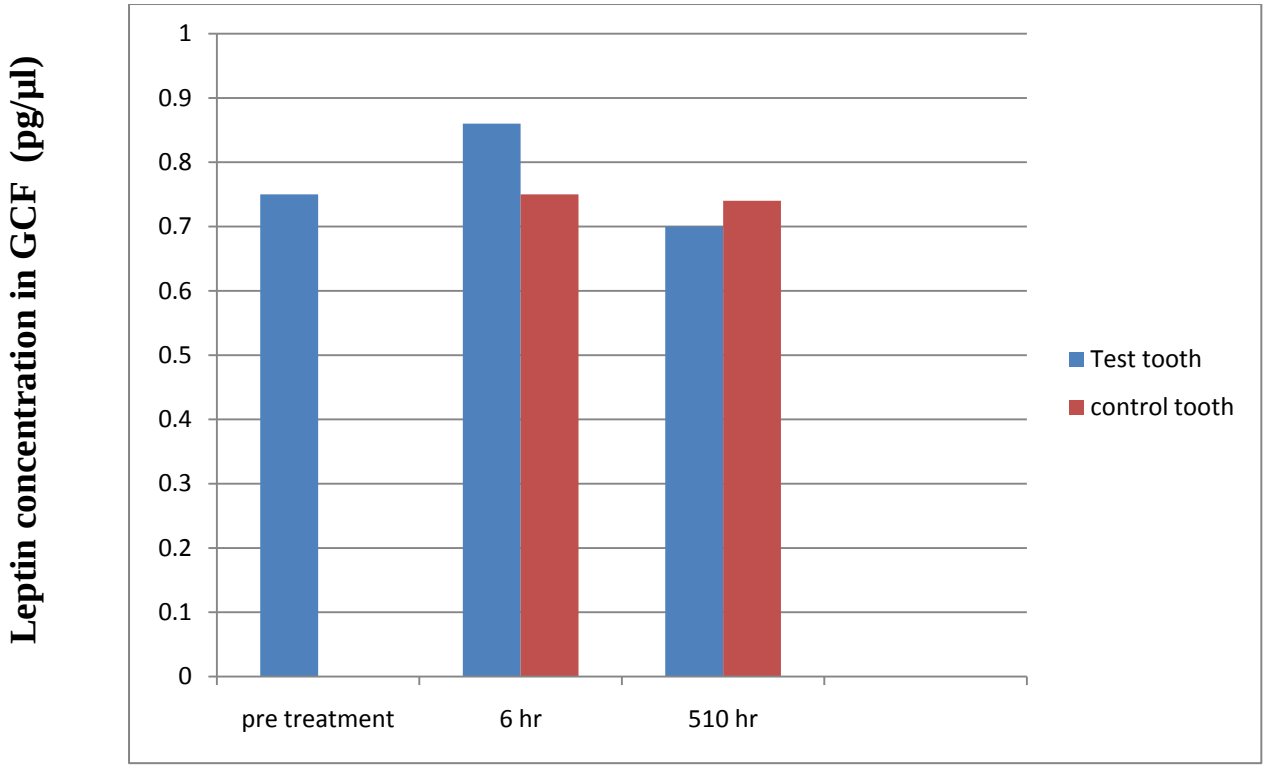


CHART - 2

Leptin levels in GCF (pg/µ l) during orthodontic tooth movement



STATISTICAL ANALYSIS

Descriptive statistics including means and standard deviations were calculated for GCF volume and GCF leptin levels of the test tooth and control tooth .One way ANOVA followed by Tukey HSD test were used. The data thus collected were assessed using SPSS statistical software.

ONEWAY ANOVA

DESCRIPTIVES

Leptin

	N	Mean	Std. Deviation	P value
Group A	25	0.7512	.11745	< 0.001**
Group B	25	0.8604	.08810	
Group C	25	0.7500	.07890	
Group D	25	0.7012	.09705	
Group E	25	0.7424	.09252	

Note : ** denotes significance of 1% level

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Leptin

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	P value	95% Confidence Interval	
					Lower Bound	Upper Bound
Group A	Group B	-.1092(*)	.02706	0.001**	-.1841	-.0343
	Group C	.0012	.02706	1.000	-.0737	.0761
	Group D	.0500	.02706	0.351	-.0249	.1249
	Group E	.0088	.02706	0.998	-.0661	.0837
Group B	Group A	.1092(*)	.02706	0.001**	.0343	.1841
	Group C	.1104(*)	.02706	0.001**	.0355	.1853
	Group D	.1592(*)	.02706	0.001**	.0843	.2341
	Group E	.1180(*)	.02706	0.001**	.0431	.1929
Group C	Group A	-.0012	.02706	1.000	-.0761	.0737
	Group B	-.1104(*)	.02706	0.001**	-.1853	-.0355
	Group D	.0488	.02706	0.376	-.0261	.1237
	Group E	.0076	.02706	0.999	-.0673	.0825
Group D	Group A	-.0500	.02706	0.351	-.1249	.0249
	Group B	-.1592(*)	.02706	0.001**	-.2341	-.0843
	Group C	-.0488	.02706	0.376	-.1237	.0261
	Group E	-.0412	.02706	0.550	-.1161	.0337
Group E	Group A	-.0088	.02706	0.998	-.0837	.0661
	Group B	-.1180(*)	.02706	0.001**	-.1929	-.0431
	Group C	-.0076	.02706	0.999	-.0825	.0673
	Group D	.0412	.02706	0.550	-.0337	.1161

* The mean difference is significant at the .05 level.

ONEWAY ANOVA

DESCRIPTIVES

GCF

	N	Mean	Std. Deviation	P value
Group A	25	.7460	.08256	<0.001**
Group B	25	.8792	.09349	
Group C	25	.7600	.07165	
Group D	25	.7308	.05986	
Group E	25	.7208	.06344	

Note ; ** denotes significance of 1% level

Post Hoc Tests

Multiple Comparisons

Dependent Variable: GCF
Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	P value	95% Confidence Interval	
					Lower Bound	Upper Bound
Group A	Group B	-.1332(*)	.02128	0 .001**	-.1921	-.0743
	Group C	-.0140	.02128	0.965	-.0729	.0449
	Group D	.0152	.02128	0.953	-.0437	.0741
	Group E	.0252	.02128	0.760	-.0337	.0841
Group B	Group A	.1332(*)	.02128	0.001**	.0743	.1921
	Group C	.1192(*)	.02128	0.001**	.0603	.1781
	Group D	.1484(*)	.02128	0.001**	.0895	.2073
	Group E	.1584(*)	.02128	0.001**	.0995	.2173
Group C	Group A	.0140	.02128	0.965	-.0449	.0729
	Group B	-.1192(*)	.02128	0.001**	-.1781	-.0603
	Group D	.0292	.02128	0.647	-.0297	.0881
	Group E	.0392	.02128	0.354	-.0197	.0981
Group D	Group A	-.0152	.02128	0.953	-.0741	.0437
	Group B	-.1484(*)	.02128	0.001**	-.2073	-.0895
	Group C	-.0292	.02128	0.647	-.0881	.0297
	Group E	.0100	.02128	0.990	-.0489	.0689
Group E	Group A	-.0252	.02128	0.760	-.0841	.0337
	Group B	-.1584(*)	.02128	0.001**	-.2173	-.0995
	Group C	-.0392	.02128	0.354	-.0981	.0197
	Group D	-.0100	.02128	0.990	-.0689	.0489

* The mean difference is significant at the .05 level.

DISCUSSION

Though there are several different biochemical analytical studies done in GCF, this study measures the levels of leptin in GCF during orthodontic tooth movement in vivo. To our knowledge, this is the first study to determine leptin levels in GCF during orthodontic tooth movement by applying **optimal force**. In the earlier study[4], tooth movement has been achieved by applying heavy magnitude of force.

Several hypotheses have been proposed to explain the biological basis of tooth movement induced by mechanical stress. Recently, the hypothesis suggesting that a mechanical stimulus causes an inflammatory response in the periodontal tissues has received considerable attention. According to this hypothesis, inflammatory mediators are released that trigger the biological processes associated with alveolar bone resorption and apposition. Thus the early phase of orthodontic tooth movement involves an acute inflammatory response, characterized by periodontal vasodilatation and migration of leucocytes out of the

capillaries[76.] These migratory cells produce various cytokines. Cytokines acting as paracrine or autocrine with other systemic and local molecules, evoke the synthesis and secretion of numerous substances by their target cells including prostaglandins, growth factors and other cytokines like leptin. These cells comprise the functional unit and remodel the paradental tissues and facilitate tooth movement [84] Remodeling changes in the alveolar bone and the PDL induce production of various cell mediators or enzymes that can be used as **biomarkers** of orthodontic treatment.

Mogi et al. [50,51] found that GCF concentrations of IL-1β and IL-6 were significantly higher in a group with severe periodontal disease compared with controls, and Yavuzyilmaz et al. [88] demonstrated the GCF IL-1β and TNF-α levels had a positive correlation to mean pocket depths, suggesting that the cytokines may be involved in the pathogenesis of periodontal diseases. Further, Mogi et al[50,51] reported that an increased concentration of RANKL and decreased concentration of OPG were detected in GCF from patients with periodontitis. Uematsu et al [82]

found that the levels of inflammatory mediators (IL-1β, IL-6, TNF-α, epidermal growth factor, and b_2 microglobulin) in GCF were elevated during orthodontic treatment, and Grieve et al. [27] reported similar results for PGE and IL-1β. Further, Lowney et al. [43] described an increase in TNF-α in GCF from teeth undergoing orthodontic forces.

Leptin is a known regulator of energy homeostasis and modulates the inflammatory response and immune system. It was shown that leptin synthesis is increased by a number of inflammatory stimuli, including IL-1, IL-6, TNF-a, and LPS.[2] An increase in leptin secretion during infection and inflammation strongly suggests that it is involved in the cytokine network that governs host defense mechanisms.[4] Leptin receptors are known to be expressed in adipocytes, T lymphocytes, and vascular endothelial cells. Leptin may have not only an indirect effect on bone metabolism via its regulation of the HPG axis and subsequent estrogen production but it may also have a direct effect on bone metabolism. Ob-Rbs have been found in primary osteoblasts and leptin has been shown in vitro to enhance differentiation of a multipotent human marrow stromal cell

line to the osteoblast. Moreover, leptin increases bone surface area in ob/ob mice implying an effect of leptin on periosteal bone deposition, but not on bone mass which seems to be decreased after leptin administration in ob/ob mice . Fetal serum leptin levels are negatively correlated with serum markers of bone resorption, suggesting a possible effect of leptin on overall increase of bone mass by decreasing bone loss. In the peripubertal period, leptin was significantly correlated with bone area but not bone mineral content, suggesting an effect of leptin on cortical bone and periosteum during puberty [22]. All these findings indicate the significant role of leptin in alveolar bone remodeling that is very important for orthodontic tooth movement.

The levels of leptin in GCF were demonstrated to be significantly lower in smokers than in non-smokers[89] .Karthikeyan and Pradeep reported that leptin concentrations in gingival crevicular fluid (GCF) were found to be higher in healthy gingiva than in tissues with periodontitis [34] Alhough there are no adipocytes in gingiva, in a recent study by Johnson & Serio[31,59] they proposed that this might be caused by entrapment of leptin within the

gingiva by diffusion from the microvasculature. As leptin has a role in the inflammatory response, an increased leptin level in healthy gingiva may be a host defense mechanism similar to that which occurs during sepsis [8.] However, during gingival inflammation the concentration of leptin is decreased as a result of expansion of the vascular network caused by vascular endothelial growth factor, which may increase the net rate of leptin removal from the gingival tissues [31,59]

Conversely, **serum leptin** levels are increased in people with periodontitis [31,59] The mechanism of these discrepancies between GCF and serum leptin levels with/without periodontitis remains unclear. One possible explanation is speculated that the secreted leptin may be used up as a substrate during inflammation.

In the earlier study by Alparslan dilslz [4] retraction of canine was done with E-chain. But studies by **Melih Y. sueri** et al[48]indicate that E- chain exerts a heavy force of 380 gm. This may lead to undermining resorption of alveolar bone there by delaying tooth movement. Moreover

E- chain gets imbibed in oral fluids and loses its force magnitude quickly before next activation. All these lead to interrupted force values.

In the present study retraction of canine was done with NiTi coil springs that deliver light constant continuous force (150-200 gm). [48] This leads to direct resorption and optimal rate of continuous tooth movement. In the current study, timing of sample collection is 6 hrs and 21 days after retractive force application with NiTi coil springs. In this study, the control tooth shows no changes in leptin levels between pretreatment, 6 hours or 21 days.

This study shows an increase in GCF leptin levels at 4-6 hours at the test tooth site that is statistically significant. This corresponds to the fact that GCF leptin levels increase in acute sepsis. When a retractive force is applied to the tooth with NiTi coil springs, acute inflammatory changes occur in the gingival and periodontal tissues. 4- 6 hours is the critical period when all the second messengers necessary for cellular differentiation and

thereby tooth movement are released into the periodontal environment.[85]

After 21 days, the test tooth with NiTi coil springs still in place shows a little decrease in GCF leptin concentration than the control side and pretreatment (Baseline) values. But this shows statistically no difference. This indicates that as the forces exerted by the NiTi coil springs are optimal (150-200 gm) unlike E- chain that exerts heavy force (380 gm), the investing tissues of the teeth recover easily and quickly with minimal hyalinization, aseptic nectrotic area and undermining resorption. In other words, gingival condition do not worsen but recovers which is indicated by returning of GCF leptin levels to normal or baseline values on the 21st day.

SUMMARY & CONCLUSION

From the findings observed in this study it can be concluded that

- When constant, continuous and optimal orthodontic forces are applied concentration of leptin in GCF is increased in early acute stages.
- When the orthodontic forces are maintained within optimal range for longer period, the gingival tissues recover quickly restoring the normal or baseline leptin values in GCF.
- Girls have more leptin concentration in GCF than boys that may be due various hormonal factors.
- Orthodontic tooth movement can be carried out without any significant destructive changes in investing tissues of the teeth provided oral hygiene is properly maintained.
- Leptin is one of the mediators of orthodontic tooth movement

Future studies are required to evaluate the levels of leptin in GCF under various force magnitudes over a long

period and to clarify the protective role of leptin in periodontal disease progression. Future interventional studies involving leptin administration are expected to further clarify the pharmaco therapeutic role of leptin in orthodontic tooth movement and periodontal disease progression.

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Annexure -1

CASE SHEET

Name: Id No:

Age / Sex:

BMI:

Gingival bleeding index:

Plaque index:

Probing depth:

Bone loss:

SAMPLES	GCF VOLUME (µl)	LEPTIN CONCENTRATION (pg/µl)
Pre treatment		
6hr test tooth		
6 hr control tooth		
21st day test tooth		
21st day control tooth		

Annexure -2

INFORMATION SHEET

STUDY TITLE

Evaluation of leptin levels in gingival crevicular fluid during orthodontic tooth movement.

PROCEDURE

Arrangement of teeth in a pleasing order requires movement of teeth within the bone. This movement results in removal of bone from one side of the tooth and deposition on the other side (remodeling).

Leptin is a hormone that is very important for this remodeling and maintenance of bone level. Leptin is present in gingival fluid. Therefore changes in the amount of leptin present in gingival fluid affects the bone level and the rate of tooth movement. If the leptin level decreases significantly during orthodontic tooth movement periodontal problems and loss of periodontal bone level may occur which in turn may affect tooth movement.

In this study, gingival fluid from each individual is collected 3 times by a non-invasive procedure

1) Before starting orthodontic treatment
2) 6 hours after applying pulling force to the upper right and left canines by using NiTi coil springs
3) 21 days after applying the pulling force

To collect gingival fluid, a **Perio paper** (a paper that absorbs the gingival fluid) is inserted into the crevice of upper left and right canine for 30 seconds and the fluid is collected non-invasively.

The volume of gingival fluid absorbed in the paper is measured and the concentration of leptin present in it is calculated using biochemical procedures.

தகவல் அறிக்கை

ஆய்வின் தலைப்பு :

பல சீரமைப்பின் போது பற்களும் ஈறுகளும் நடுவிலிருந்து வெளிப்படும் நீரிலுள்ள லெப்டின் அளவை கணக்கிடுதல்.

செய்முறை :

பற்களை அழகிய வரிசையில் சீரமைப்பதற்கு, எலும்பினிடையே பற்கள் நகர்வது அவசியம். இவ்வாறு பற்கள் நகர்வதற்கு, பற்கள் சுற்றியுள்ள எலும்பு ஒரு புறம் நீக்கப்பட்டு, மறுபுறம் கட்டப்படுவது அவசியம் (ரீமாடலிங்)

இவ்வாறு பற்கள் சுற்றியுள்ள எலும்பு சீர் அமைவதற்கும், எலும்பின் உயரம் பாதுகாக்கப்படுவதற்கும், "லெப்டின்" எனப்படும் சுரப்பி மிக அவசியம். இந்த "லெப்டின்" பற்களுக்கும், ஈறுகளுக்கும் நடுவிலிருந்து புறப்படும் திரவத்தில் அமைந்துள்ளது. எனவே இந்த நீரில் ஏற்படும் "லெப்டின்" அளவின் மாற்றம் பற்களைச் சுற்றியுள்ள எலும்பையும், பற்கள் நகர்த்தப்படும் வேகத்தையும் பாதிக்கக்கூடும். பற்கள் நகர்த்தப்படும்போது, இந்த நீரில் உள்ள "லெப்டின்" அளவு குறைந்தால் ஈறு நோய் மற்றும் பற்களை சுற்றியுள்ள எலும்பின் உயரம் குறைதல் போன்ற பிரச்சினைகள் ஏற்பட வாய்ப்பு உண்டு.

இந்த ஆய்வில் ஒவ்வொரு நபரிடமிருந்து மூன்று முறை இந்த திரவம் சேகரிக்கப்படுகிறது.

1.பல் சீரமைப்பு சிகிச்சை துவங்கும் முன்

2. பல் சீரமைப்பு சிகிச்சையின் போது மேல்தாடை கோரைப்பற்களை நைட்டை சுருள் ஸ்பிரிங் உதவி கொண்டு பின்னாக இழுக்கும் விசை கொடுக்கப்பட்ட ஆறு மணி நேரத்திற்குபின்

3. பின்னாக இழுக்கும் விசை கொடுக்கப்பட்ட 21 நாள்களுக்கு பின்

இந்த திரவத்தை சேசகரிப்பதற்கு, பெரியோ பேப்பர் (பற்களுக்கும், ஈறுகளுக்கும் நடுவிலிருந்து புறப்படும் நீரை சேகரிக்கும் தன்மையுடைய தாள்). மேல்தாடையின் வலது மற்றும் இடது கோரைப்பற்களுக்கும், ஈறுகளுக்கும் நடுவில் உள்ள குழியில் 30 வினாடிகள் வைக்கப்படுகிறது. இந்த தாளில் சேகரிக்கப்படும் திரவத்தின் அளவும், அந்த திரவத்தில் உள்ள "லெப்டின்" அடர்த்தியும் உயிர் வேதியியல் முறையில் கணக்கிடப்படுகிறது.

Annexure -3

INFORMED CONSENT FORM

STUDY TITLE

EVALUATION OF LEPTIN LEVELS IN GINGIVAL CREVICULAR FLUID DURING ORTHODONTIC TOOTH MOVEMENT

Name: O.P:

Address: Code No.:

Tel. No.: Age/ Sex:

I, _____ , exercising my free power of choice, hereby give my consent to be included as a participant in the study.

I agree to the following:

* I have been informed to my satisfaction about the purpose of the study and study procedures.

* I have fully understood the study procedure and I am aware of the noninvasive procedures to be done

* I agree to cooperate fully and to inform my doctor immediately if I suffer any unusual symptom.

* I agree that the orthodontic procedure may be used for the research purpose.

* I agree to report to the doctor for a regular follow up as and when required for the research.

* I have informed the doctor about all medications that I am currently taking and other systemic illness that I have.

* I hereby give permission to use my medical records for research purpose. I am told that the investigating doctor and institution will keep my identity confidential.

Name of the patient Name of the investigator

Signature / Thumb impression Signature

Date :

ஒப்புதல் படிவம்

ஆய்வின் தலைப்பு

பல் சீரமைப்பு சிகிச்சையின்போது ஈறுகளுக்கும் பற்களுக்கும் நடுவிலிருந்து வெளிப்படும் திரவத்தில் லெப்டின் அளவை கணக்கிடுதல்.

பெயர்	:	ஓ.பி. நம்பர்	:
முகவரி	:	குறியீட்டு எண்	:
தொலைபேசி எண்	:	வயது / பாலினம்	:

_____ஆகிய நான் என்னை இந்த ஆய்விற்கு உட்படுத்துவதற்கு முழுமையாக சம்மதிக்கிறேன்.

நான் கீழ்கண்டவற்றிற்கு முழுமையாக ஒப்புக்கொள்கிறேன்.

- ஆய்வின் அவசியமும், ஆய்வின் முறைகளும் எனக்கு புரியும்படி முழுமையாக தெரிவிக்கப்பட்டுள்ளது.
- உடலை ஊடுருவாமல் எனக்கு செய்யப்படும் ஆய்வின் முறைகளை நான் முழுமையாக புரிந்துக் கொண்டேன்.
- ஆய்வில் முழுமையாக ஒத்துழைப்பதற்கு நான் சம்மதிக்கிறேன்.
- ஆய்வின் போது எனக்கு ஏதேனும் அசாதாரண பிரச்சினைகள் ஏற்பட்டால் உடனடியாக எனது மருத்துவரிடம் தெரிவிப்பதற்கு சம்மதிக்கிறேன்.
- எனது பல் சீரமைப்பு முறைகளை ஆய்விற்கு பயன்படுத்த சம்மதிக்கிறேன்.
- நான், ஆய்விற்கு தேவைப்படும் போதெல்லாம் மருத்துவமனைக்கு வருவதற்கு சம்மதிக்கிறேன்.
- நான் தற்சமயம் உட்கொள்ளும் மற்றும் உபயோகிக்கும் மருந்துகள் சம்மந்தமான எல்லா விவரங்களையும் எனது மருத்துவரிடம் தெரிவித்துள்ளேன்.
- எனது சிகிச்சை குறித்த விவரங்களை ஆய்விற்கு பயன்படுத்துவதற்கு சம்மதம் தெரிவிக்கிறேன்.
- எனது சிகிச்சை குறித்த விவரங்கள் இரகசியம் காக்கப்படும் என்று உறுதி அளிக்கப்பெற்றுள்ளேன்.

நோயாளி / பெற்றோர் பெயர்: ஆய்வாளரின் பெயர் :

கையொப்பம் / பெருவிரல் ரேகை: கையொப்பம் :